



Uptake and localization of fluorescent labelled gold nanoparticles in living zebrafish (Danio rerio) using Light Sheet Microscopy

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MO320 Uptake and localization of fluorescent labelled gold nanoparticles in living zebrafish (*Danio rerio*) using Light Sheet Microscopy. L.M. Skjolding, DTU / DTU Environment; G. Asmunaite, University of Gothenburg / Department of Biological and Environmental Sciences; R. Jolk, DTU Technical University of Denmark / Department of Micro and Nanotechnology; A. Baun, Technical University of Denmark DTU / DTU Environment; J. Sturve, Goteborg University / Zoophysiology Dept. Despite nanoparticles being used in many different products and applications, the effects and fate in the environment are still not well understood. Uptake of nanoparticles into cells has been shown in vitro and in vivo. However, it is challenging to find suitable methods to identify uptake and determine localization on a whole organism level. Furthermore, methods used to identify nanoparticle uptake have been associated with artefacts induced by sample preparation including staining methods for electron microscopy. This study used Fluorescent Light Sheet Microscopy (FLSM) to determine uptake and localization of fluorescent labelled nanoparticles in living whole organisms with minimal sample preparation. Two strains of *D. rerio* (wildtype AB and transparent Casper) were exposed to 50 nm PEG coated gold nanoparticles (Au NP) synthesized with 1% of a fluorescent probe (FITC). The fish were exposed to the particles through the diet or the water phase in a series of separate experiments. In the dietary exposure experiments *Artemia salina* were exposed to 1 mg Au/L for 24h before being fed to *D. rerio*. For exposure through the water phase 1 mg Au/L was added directly to aquaria holding the fish and non-exposed *A. salina* was used for feeding. Imaging of *D. rerio* was done after 1, 3 and 7d of uptake and again after 1 and 3d of depuration with FLSM for both dietary and aqueous exposures. Though the FLSM proved to be excellent for in vivo detection of NP, it was found that the opaqueness of wildtype AB zebrafish hindered deep penetration of the light thus yielding less clear images compared to the Casper strain. A time dependent increase in fluorescence was observed in the gut region of the fish after dietary exposure. No fluorescence was observed outside the gut channel indicating no or limited transfer of AuNP outside the gut region. Fish exposed through the water phase showed adherence of AuNP to fins and especially gills. Strong fluorescence signal was also observed in guts of fish due to passive intake or drinking of exposed water. Fluorescence was also detectable in parts of the head which could indicate uptake to the brain through the olfactory tract. During the depuration phase the fluorescent signal decreased with time for both exposure scenarios. These results suggest that the route of uptake is pivotal for localization and transport of nanoparticles in zebrafish. Furthermore, the study shows the suitability for whole imaging of living organisms using FLSM.